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Restoring and Maintaining Bone in Osteopenic Female Rat Skeleton: I. Changes in Bone Mass and Structure

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ABSTRACT

This experiment contains the crucial data for the lose, restore, and maintain (LRM) concept, a practical approach for reversing existing osteoporosis. The LRM concept uses anabolic agents to restore bone mass and architecture (+ phase) and then switches to an agent with the established ability to maintain bone mass, to keep the new bone (± phase). The purpose of this study was to learn whether switching to an agent known chiefly for its ability to maintain existing bone mass preserves new bone induced by PGE_2 in osteopenic, estrogen-depleted rats. The current study had three phases, the bone loss (−), restore (+), and maintain (±) phases. We ovariectomized (OX) or sham ovariectomized (sham-OX) 5.5-month-old female rats (− phase). The OX rats were treated 5 months postovariectomy with 1-6 mg PGE_2 per kg/day for 75 days to restore lost cancellous bone mass (+ phase), and then PGE_2 treatment was stopped and treatment began with 1 or 5 μg/kg of risedronate, a bisphosphonate, twice a week for 60 days (± phase). During the loss (−) phase, the cancellous bone volume of the proximal tibial metaphysis in the OX rat fell to 19% of initial and 30% of age-matched control levels. During the restore (+) phase, the cancellous bone volume in OX rats doubled. When PGE_2 treatment was stopped, however, and no special maintenance efforts were made during the maintain (±) phase, the PGE_2-induced cancellous bone disappeared. In contrast, the PGE_2-induced cancellous bone persisted when the PGE_2 treatment was followed by either a 1 or 5 μg treatment of risedronate per kg given twice a week for 60 days during the maintain (±) phase. The tibial shaft demonstrated very little cortical bone loss during the loss (−) phase in OX rats. The tibial shaft cortical bone fell some 8%. During the restore (+) phase, new cortical bone in OX rats increased by 22%. When PGE_2 treatment was stopped and nothing was given during the maintain (±) phase, however, all but the PGE_2-induced subperiosteal bone disappeared. In contrast, when PGE_2 treatment was stopped and 1 μg risedronate per kg twice a week for 60 days was administered during the maintenance (±) phase, the PGE_2-induced subperiosteal bone and some of the subendocortical bone and marrow trabeculae persisted. When 5 μg risedronate per kg was given twice a week, all the PGE_2-induced bone persisted. The study shows that most of the new cancellous and cortical bone induced by PGE_2 can be maintained for at least 60 days after discontinuing PGE_2 by administering enough of the resorption inhibitor, risedronate. The lower dose of risedronate was not adequate to save most of the PGE_2-induced endocortical bone.

INTRODUCTION

PERMANENTLY RAISING BONE MASS and improving the bone structure of osteoporotic subjects seems the ideal way to cure osteoporosis. Saving the remaining bone in a fracturing person seems a poor second choice. Although adding new bone at *any* site should benefit osteoporotic subjects, the best way to increase bone bending strength is to add bone at its periosteal and endocortical surfaces. Periosteal bone contributes more to bending strength than a similar quantity located nearer its central axis.[1-6] Increasing endocortical bone could improve cancellous bone quality.[7,8] A treatment that did both would be ideal.

One possible approach for reversing existing osteopenia

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is a two-step treatment called lose→restore→maintain (LRM). It includes the following steps: (1) use a bone anabolic agent to *restore* lost bone (+ phase), and then (2) switch to an agent known to *maintain* existing bone mass, to keep the new bone (± phase).

The use of a bone anabolic agent during the restore (+) phase is necessary because osteoporosis subjects usually have low bone mass and increased fracture risk at many sites, not only at those that exhibit low trauma fracture. In animal experiments, bone anabolic agents during a successful restore (+) phase induce a finite increase in bone mass that then disappears after cessation of treatment.[9-11] Switching to a maintenance agent during the maintain (±) phase is appealing because effective bone formation stimulators are now scarce, expensive, and deliverable only parenterally.

Earlier studies showed that prostaglandin E_2 (PGE_2) is a suitable agent for the restore (+) phase of testing the LRM concept. Although the vigorous in vivo hard tissue positive bone balance effects of PGE_2 seem different from some of its in vitro effects,[12] the anabolic effect data from dogs, humans, and rats is compelling.[13] PGE_2 increases collagen synthesis and prolyl hydroxylase activity in cloned osteoblasts[14] and reverses cortisol-inhibited bone growth.[15] Woven periosteal bone has formed in human infants given PGE_1[16,17] and in dogs and rats given PGE_2.[5-8,18,19] PGE_2 promotes bone remodeling in both dogs[20,21] and rats.[5-8,22,24] It stimulates the formation of new woven bone trabeculae in the marrow cavities of tibial metaphyses and diaphyses and adds new bone at the diaphyseal endocortical and periosteal surfaces,[5-8,23-26] an ideal place to strengthen bone.[1-4] It increases bone mass and adds new trabeculae in the bone-depleted proximal tibial metaphysis and vertebral spongiosa of the osteopenic, ovariectomized (OX) rat.[7,26] During continued administration a plateau phase develops, after which there is no further increase in bone.[6,8] Its continued administration is currently the only way to preserve the new bone it induces, however, since PGE_2-induced bone rapidly disappears upon treatment cessation.[10,11,27,28]

Agents that can maintain existing bone mass include estrogen, bisphosphonates, calcitonin, androgens, and calcium.[29-44] The data seem most compelling for the first three. We chose risedronate, a third-generation bisphosphonate.

This report contains a study of the LRM concept for treating established osteopenia to discover whether switching to an agent known to maintain existing bone mass could preserve new bone induced by PGE_2 in osteopenic, estrogen-depleted rats.

MATERIALS AND METHODS

Animal care and study protocol

A total of 53 virgin Sprague-Dawley female rats aged 5.5 months and weighing 260 g were acclimated to local vivarium conditions for 2 weeks. Animals were housed in single cages (21 × 32 × 20 cm^3) and allowed free access to a pelleted commercial diet (Rodent Chow 5001; Ralston-Purina Co., St. Louis, MO), which contains 1.46% calcium and 0.99% phosphorus, and 4.96 IU/g of vitamin D_3 and water. Rats were weighed weekly during the first month and every 2 weeks thereafter.

This experiment had three phases: bone loss (−), bone gain (+), and bone maintenance (±). We ovariectomized or sham-OX the 5.5-month-old female rats for 150 days, the loss (−) phase. We treated osteopenic, OX rats for 76 days with 1-6 mg PGE_2 per kg/day to restore lost bone mass, the restore (+) phase. We then stopped PGE_2 treatment and began treatment with 1 or 5 μg/kg of risedronate twice a week for 60 days, the maintain (±) phase. The report deals with the fate of cancellous and cortical bone during these phases as shown by morphometric analysis of microradiographs of the proximal tibial metaphyses and diaphyses. Dynamic morphometric analysis follows.

On the first day of the study, six rats were sacrificed as baseline (basal or beginning) controls; others were ovariectomized (OX, 35 rats) and sham ovariectomized (sham, 12 rats). A total of 4 animals from sham and 4-5 animals from OX were sacrificed at 5, 7.5, and 9.5 months after operation as controls to monitor the aging and OX skeletal changes. At 5 months after operation, all other OX rats in the loss (−) phase received PGE_2 treatment. For the first 2 weeks they received lower doses of PGE_2 (1 mg/kg for 4 days, 2 mg/kg for 4 days, and 3 mg/kg for 8 days) to accustom them to the PGE_2 and then 6 mg/kg daily for 60 days, the restore (+) phase. A total of 5 rats were sacrificed at the end of the treatment period (7.5 month postoperation) to assess the amount of bone restored. Then, all rats that received PGE_2 treatment were divided into three groups. The first group (5 rats) received only sterile normal saline injection; the remaining two groups (5 rats in each) were treated with risedronate, a bisphosphonate compound (Procter and Gamble Pharmaceuticals, Inc., Cincinnati, OH) in doses of 1 and 5 μg/kg twice a week for 60 days, respectively, the maintain (±) phase. They were sacrificed after 60 days of treatment (9.5 months postoperation at 15 months of age).

PGE_2 injections were prepared the same way as before.[5-8] Risedronate was prepared in saline at concentrations of 0 (vehicle), 0.5, and 2.5 μg/ml. Each animal was administered 2 ml/kg body weight of the appropriate solution, subcutaneously on the back.

All rats were sacrificed by cardiac puncture under ketamine hydrochloride and xylazine anesthesia. The left tibia was removed, dissected free of soft tissue, and cut into three equal parts. The proximal third was trimmed frontally using a low-speed metallurgical saw (Buehler Ltd., Lake Bluff, IL) to expose the marrow cavity for better fixation. After 24 h fixation in 70% alcohol, the left proximal tibia and tibial shaft were immersed in Villanueva bone-staining solution (Poly-sciences, Inc., Warrington, PA) for 4 days. The specimens were dehydrated by sequential changes of ascending concentrations of ethanol and acetone and then embedded in methyl methacrylate (Eastman Kodak Chemicals, Rochester, NY). Frontal sections of the proximal tibia and two cross sections of the tibial shaft proximal to the tibiofibular junction were cut at 230 μm using a low-speed metallurgical saw (Buehler Ltd., Lake

TABLE 1. HISTOMORPHOMETRY PARAMETERS

Parameters	*Abbreviation*	*Comment or formulas*	*Unit*
Proximal tibial metaphysis			
Measured parameters			
Tissue area	T.Ar.	Measured tissue area between 0.5 and mm distal to growth plate-metaphyseal junction	mm^2
Trabecular bone area	Tb.Ar	Combined area of woven and lamellar bone	mm^2
Trabecular bone perimeter	Tb.Pm.	Combined surface of woven and lamellar bone	mm
Number of nodes	Node no.	Number of joining points by two or more trabeculae	No.
Free end to free end	FTF	Number of trabeculae not connected to others in both ends	No.
Node to free end	NTF	Number of trabeculae with only one end connected to node	No.
Node to node	NTN	Number of trabeculae with both ends connected to nodes	No.
Cortical bone to free end	CTF	Number of trabeculae with one end connected to cortical bone	No.
Cortical bone to node	CTN	Number of trabeculae with one end to node and another to cortex	
Derived parameters			
Percentage trabecular bone area	% Tb.Ar.	Tb.Ar/T.Ar × 100	%
Trabecular bone thickness	Tb.Th	(2000/1.199) × Tb.Ar/Tb.Pm	μm
Trabecular bone number	Tb.N	(1.199/2) × Tb.Pm/T.Ar	No./mm
Trabecular bone separation	Tb.Sp	(2000/1.199) × (T.Ar − Tb.Ar)/Tb.Pm	μm
Node density/BV	Node no./BV	Node no./Tb.Ar.	No./mm^2
Density of free to free/BV	FTF/BV	No. of FTF/Tb.Ar	No./mm^2
Density of node to free/BV	NTF/BV	No. of NTF/Tb.Ar	No./mm^2
Density of node to node/BV	NTN/BV	No. of NTN/Tb.Ar	No./mm^2
Density of cortical to free/BV	CTF/BV	No. of CTF/Tb.Ar	No./mm^2
Density of cortical to node/BV	CTN/BV	No. of CTN/Tb.Ar	No./mm^2
Node density/TV	Node no./TV	Node no./T.Ar.	No./mm^2
Density of free to free/TV	FTF/TV	No. of FTF/T.Ar	No./mm^2
Density of node to free/TV	NTF/TV	No. of NTF/T.Ar	No./mm^2
Density of node to node/TV	NTN/TV	No. of NTN/T.Ar	No./mm^2
Density of cortical to free/TV	CTF/TV	No. of CTF/T.Ar	No./mm^2
Density of cortical to node/TV	CTN/TV	No. of CTN/T.Ar	No./mm^2
Tibial diaphysis			
Measured parameters			
Total tissue area	T.Ar	Measured whole tissue area	mm^2
Marrow cavity area	Ma.Ar	Marrow cavity area	mm^2
Marrow trabecular bone area	Ma.Tb.Ar	The hard tissue area in marrow cavity	mm^2
Derived parameters			
Percentage total bone area	%TB.Ar	(Ct.Ar + Ma.Tb.Ar)/T.Ar. × 100	%
Percentage marrow area	%Ma.Ar.	(T.Ar. − M.Ar. − Ma.Tb.Ar)/T.Ar × 100	%
Percentage marrow trabecular bone area	%Ma.Tb.Ar	Ma.Tb.Ar/T.Ar × 100	%
Percentage restoration Tb.B (trabecular bone)	% Restoration	(Value of PGE_2 − value of Ovx)/(value of aging − value of Ovx) × 100	%
Percentage restoration and addition Ct.B (cortical bone)	% Restoration and addition	(Value of PGE_2 − value of Ovx)/(value of aging − value of Ovx) × 100	%

Bluff, IL) and then ground to 100 μm for microradiographs using a precision lapping machine (Maruto Co., Tokyo, Japan). Microradiographs were taken on Kodak high-resolution plates (IMTEC, Sunnyvale, CA) at 12 kV and 25 mA for 7 minutes for proximal tibia and 10 minutes for tibial shaft.[45]

Microradiograph analysis of proximal tibial metaphyses

A video image analysis system (VIAS) was used for microradiographic analysis of the proximal tibial metaphysis (PTM). The VIAS consisted of a quantitative television microscope (SZ-CTV; Olympus, Japan) coupled with a CCTV camera (WV-BD 400; Panasonic, Japan) and an Apple Macintosh IIsi Computer (Apple Computer, Inc., Santa Clara, CA). Using the KSS Image Analysis program (KSS Scientific Consultants, Magna, UT), measurements of trabecular bone mass and microanatomic structure were performed in the area beginning 0.5 mm distal from the growth cartilage metaphyseal junction (GCMJ) and extending distally to 3.5 mm. The 0.5 mm metaphyseal region was omitted to restrict measurements to the secondary spongiosa.[46] Total tissue area, trabecular bone area, and perimeter were measured directly, and percentage trabecular bone area, trabecular number, width, and separation were calculated according to Jee et al.[45] and Parfitt et al.[47,48] The indices of the secondary spongiosa's microanatomic structure, which included the number of trabecular nodes, node to node, node to free end, free end to free end, and cortical to node, were measured according to Garrahan et al.[49–51] and were normalized to total tissue and trabecular bone areas to calculate their density (Table 1). These indices provided data on the interconnectedness of the trabecular bone structure and on the number of structural elements. Node to node and cortical to node indicated interconnectedness; free to free end, node to free end, and cortex to free end represented lack of interconnectedness. When the trabecular or struts were connected, the node to free end ratio was high, but it was low when the structures were broken.

Static measurements of tibial diaphyses

Using microradiographs of the tibial shaft sections, a digitizing image system was used to determine perimeter in the cortical tissue, marrow cavity, and marrow trabecular bone areas. The system consisted of a digitizing pad (CR^{TM}1212, Fairfield, CT) coupled to a computer (Apple Macintosh SE; Apple Computer, Inc., Cupertino, CA) with the morphometry program Stereology (KDS Scientific Consultants, Magna, UT). These measured parameters were used to calculate the derived parameters in Table 1, according to Jee et al. and Parfitt et al.[45,48]

Statistical analysis

All data were expressed as means ± standard deviation of the means. Analysis of variance was used to evaluate the significant difference among group means in each time period. When the analysis of variance indicated significant differences among means, the differences were evaluated using Dunnett's *t*-test.[52] The differences between the means from different time periods were analyzed using the two-tailed Student's *t*-test. Probability <0.05 was considered significant.

RESULTS

Body weight changes with aging, OX, and during LRM treatment

Figure 1 shows the body weight changes associated with age and OX with and without PGE_2 and risedronate treatment. As expected, the OX rats between 0 and 2 months post-OX gained some 14–20% more than the sham-OX and plateaued at that weight level throughout the study.[53,54] When PGE_2 was administered to the OX rat, there was a 8% drop in the body weight compared to the control OX rats, but the rats were still 10% heavier than sham OX rats. The body weight of the PGE_2-treated rat returned to the untreated OX rat level when the PGE_2 treatment was terminated.

Cancellous bone changes in the proximal tibial metaphyses with aging, OX, and LRM treatment

Effects of Aging: Figures 2 through 5 show that the cancellous bone mass and structures reached a new steady state (equilibrium) of less bone area (−37%) and poorer structure (thickness, −20%; number, −22%; and separation, 55%) from 10.5 months of age onward (Fig. 3). No significant changes in bone mass and structural parameters were observed between 10.5 and 15 months of age, indicating that these parameters had plateaued. Tissue-based node density, node to node and cortical to node decreased,

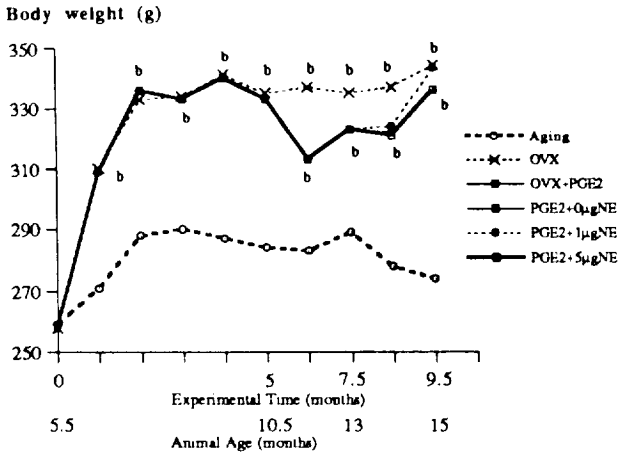

FIG. 1. Body weight changes in aging controls and OX rats treated with and without PGE_2 and risedronate. Experimental time from 0 (animal age is 5.5 months) to 9.5 months (animal age is 15 months). $^bP < 0.05$ compared to aging control group.

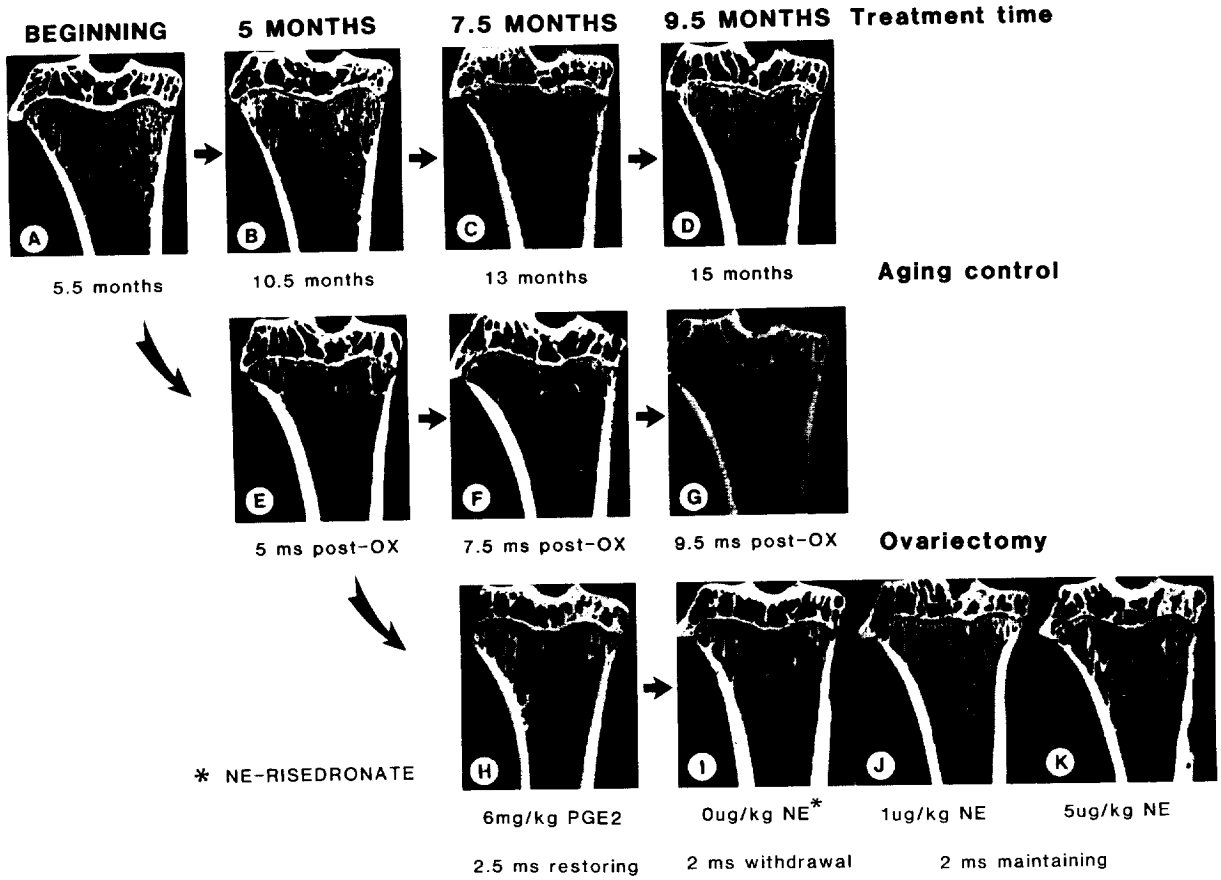

FIG. 2. Microradiographs of cancellous bone mass and structural changes in the PTM with aging and OX with and without PGE_2 and risedronate treatment. (A–D) Changes in aging rats; (E–G) bone loss during 5–9.5 months post-OX; (H) restoration of bone after 2 months of PGE_2 treatment; (I–K) changes during the maintenance phase without (I) and with 2 months of risedronate treatment (J and K). (×4)

but bone-based free to free end and cortical to free end increased at 10.5 months of age and plateaued thereafter (Figs. 4 and 5).

Effects of Ovariectomy: Cancellous Bone Loss (−) Phase: Figures 2 through 5 show that during the first 5 months after OX, the trabecular bone area, thickness, and number reached a new steady state of less cancellous bone (−70%) and poorer structure. The tissue-based microanatomic structures all decreased and established a new steady state from that of basal and aging controls by 5 months post-OV. The bone-based trabecular changes differed from those based on tissue area: the densities of free to free and cortical to free ends established a new steady state of increased free ends at 5 and 7.5 months post-OV compared to basal and aging controls.

Effects of PGE_2 Treatment: The Restoration (+) Phase: Figures 2 through 5 show that 76 days of PGE_2 administration to the 5 month post-OX rats partially restored (52%) the cancellous bone mass in the PTM that was lost as a result of OX. The percentage of trabecular bone area increased 189% over the OX level but was still 39% below that of the aging control rats. In addition, trabecular thickness increased 45% over OX and 29% over aging controls and trabecular number increased 11% over OX but decreased by 52% of basal control levels.

PGE_2 treatment restored the tissue-based trabecular node density (32%) and the densities of node to node (43%), cortical to node (67%), and cortical to free end (17%); the bone-based trabecular structure showed more increase in the same parameters along with a decrease in free to free ends (−118%).

Effects of PGE_2 Withdrawal and the ± Addition of Risedronate: Maintenance (±) Phase: Figures 2 through 5 show the effects of no treatment and risedronate treatment following discontinuation of PGE_2. The no treatment rats lost bone mass, and the bone structure deteriorated. However, rats treated with risedronate maintained the new bone mass and structure.

The withdrawal of PGE_2 without further treatment resulted in the loss of PGE_2-induced bone mass and most of its structure to the OX level. Only tissue-based cortical to free end (216%) and bone-based cortical to node (260%)

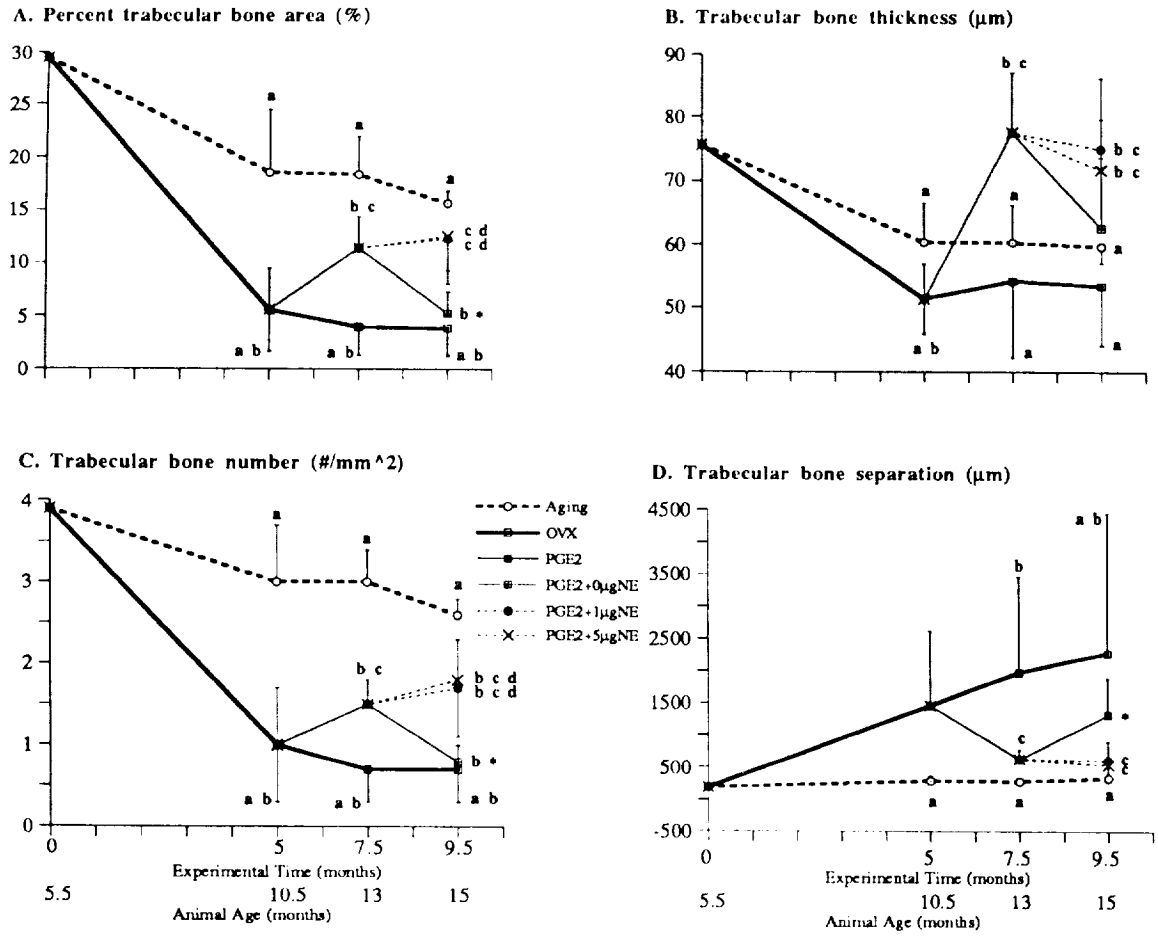

FIG. 3. Cancellous bone mass and structural changes in the PTM in aging controls and OX rats treated with and without PGE_2 and risedronate. Experimental time and animal age are the same as in Fig. 1. $P < 0.05$ compared to basal (a), aging (b), OX (c), PGE_2 + 0 μg NE (d), and PGE_2-treated group (*).

and free end (80%) were elevated over OX levels (Figs. 2 through 5).

The additon of 1 and 5 μg risedronate per kg, twice a week for 60 days after the termination of PGE_2 treatment, maintained the bone mass and structure at the post-PGE_2 treatment level. The tissue- and bone-based trabecular microanatomic parameters were maintained at the PGE_2 restoration level. The tissue-based density of free to free end decreased significantly, but others were not significantly different from age-related control levels (Figs. 2 through 5).

Tibial diaphyses changes with aging, ovariectomy, and LRM treatment

Effects of Aging: Figures 6 and 7 show the only parameter affected by aging was total tissue or cross-sectional area, which increased 11% at age 13 months compared to 5 months of age.

Effects of Ovariectomy: Cortical Bone Loss (−) Phase: Figures 6 and 7 show that OX increased total or cross-sectional and marrow areas but decreased total bone areas compared to basal controls. By 9.5 months post-OX, the total area had increased a significant 11%. Similarly, the marrow area increased by 25, 43, and 46% at 5, 7.5, and 9.5 months post-OX, but total bone area decreased by 6, 10, and 11% for the same periods. Compared to the sham-OX or aging controls, there was 8–9% less total bone area and 30–39% more marrow area at 5, 7.5, and 9.5 months post-OX. The occurrence and magnitude of these changes indicate that a new steady state of less bone was established by no later than 7.5 months post-OX.

Effects of PGE_2 Administration: Restoration and Addition (+) Phase: Figures 6 and 7 show that 2.5 months of PGE_2 treatment in 5 month post-OX rats added extra bone to the total tissue or cross-sectional areas by 15% and the percentage of total bone area by 11%. It reduced the percentage of marrow space by some 45% and added 479% in percentage of marrow trabeculae compared to aging or sham-OX control values. Compared to the OX animals, these changes were slightly larger. The large reduction in marrow space was due mainly to filling of the marrow cavity with new endocortical and marrow trabecular bone.

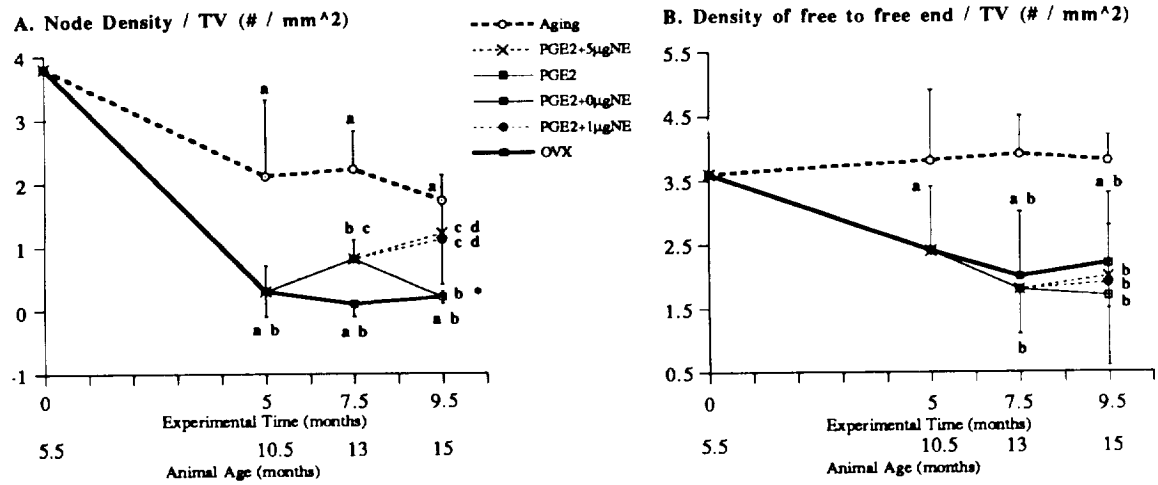

FIG. 4. Microanatomic strut changes based on tissue area in the PTM in aging controls and OX rats treated with and without PGE_2 and risedronate. All other plots, NTN/TV, NTF/TV, CTN/TV, and CTF/TV, are similar to the plot of node density/TV. Experimental time and animal age are the same as in Fig. 1. $P < 0.05$ compared to basal (a), aging (b), OX (c), PGE_2 + 0 μg NE (d), and PGE_2-treated group (*).

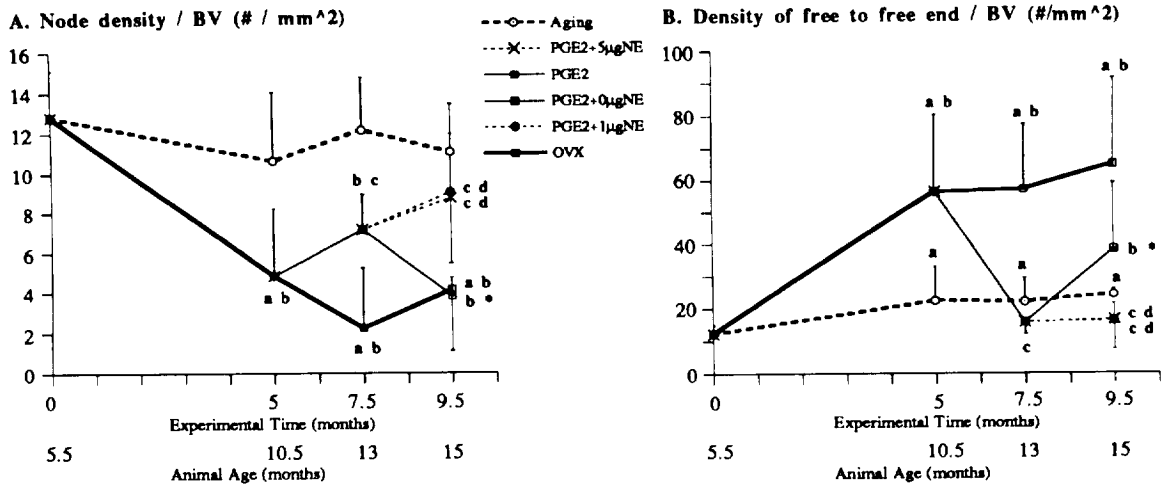

FIG. 5. Microanatomic strut changes based on trabecular bone area in the PTM in aging and OX rats treated with and without PGE_2 and risedronate. The plot of CTF/BV is similar to the plot of FTF/BV. Other plots, NTN/BV, NTF/BV, and CTN/BV, are similar to the plot of node density/BV. Experimental time and animal age are the same as in Fig. 1. $P < 0.05$ compared to basal (a), aging (b), OX (c), PGE_2 + 0 μg NE (d), and PGE_2-treated group (*).

Effects of PGE_2 Withdrawal and the ± Addition of Risedronate: Maintenance (±) Phase: Figures 6 and 7 show the results of withdrawal of PGE_2 treatment with the addition of an antiresorptive agent, risedronate. Withdrawal of PGE_2 without further treatment for 2 months resulted in the loss of the endocortical and marrow trabecular bone induced by PGE_2 that enlarged the marrow cavities to the age-related control level. However, the resulting tibial shaft retained the added subperiosteal bone to increase the total tissue or cross-sectional area by 14% of aged and 10% of OX control values.

The maintenance of cortical bone by 1 μg risedronate per kg twice a week after PGE_2 withdrawal produced a tibial shaft that partially maintained the added bone induced by PGE_2. It could not maintain all the marrow trabecular bone induced by the PGE_2 treatment (−72% of OX PGE_2 and a nonsignificant 17% of aging control values). Nevertheless, there was 16% more total tissue, a 6% increase in percentage of total bone, and a 20% decrease in marrow areas compared to age-matched controls.

Risedronate at 5 μg/kg given twice a week for 60 days after the withdrawal of PGE_2 maintained the added bone mass induced by PGE_2 during the restoration phase. The resulting bone exhibited a 13% increase in percentage of total tissue; 14% in total bone area; 374% in percentage of marrow trabecular bone; 14% in percentage of total bone

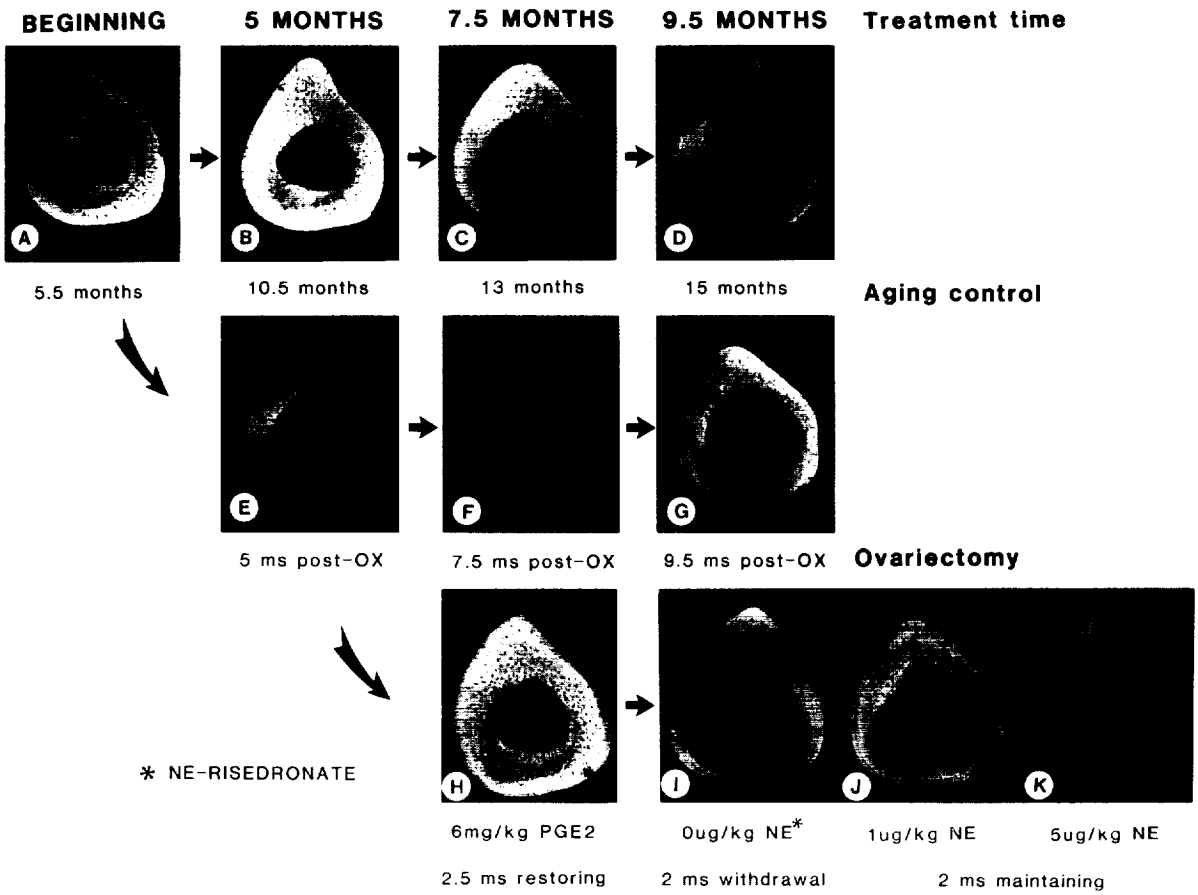

FIG. 6. Microradiographs of tibial diaphysis with aging and OX with and without PGE_2 and risedronate treatment. (A-D) Changes in aging rats; (E-G) changes during 5-9.5 months post-OX, the loss phase; (H) change of restoration and addition of bone after 2 months of PGE_2 treatment; (I-K) changes during the maintenance without (I) and with 2 months of risedronate treatment (J and K). (×8)

area; and a 55% decrease in percentage of marrow compared to aging controls.

DISCUSSION

This experiment contains the crucial data for the lose, restore, and maintain concept. The purpose was to learn whether switching to an agent known chiefly for its ability to maintain existing bone mass could preserve new bone induced by PGE_2 in osteopenic, estrogen-depleted rats. This experiment had three phases: lose (−), restore (+), and maintenance (±) bone phases. To induce bone loss, we OX or sham-OX 5.5-month-old female rats, the loss (−) phase. During the loss (−) phase, cancellous bone volume in the OX rat fell to 21% of initial and 33% of age-matched control values (Fig. 2E versus B and A). We treated osteopenic, OX rats for 75 days with 1-6 mg PGE_2 per kg/day to restore lost cancellous bone mass, the restore (+) phase. During this phase, the cancellous bone volume in OX rats doubled (Fig. 2H versus E). When PGE_2 treatment was stopped, however, and no special maintenance efforts were made during the maintain (±) phase, the PGE_2-induced cancellous bone disappeared (Fig. 2I versus H). In contrast, when PGE_2 treatment was stopped but either dose of risedronate treatment was given during the maintain (±) phase, the PGE_2-induced cancellous bone persisted (Fig. 2J and K versus H and I). This LRM trial indicates that new cancellous bone with better structure induced by PGE_2 can be maintained for at least 60 days after discontinuing PGE_2 by treatment with a resorption inhibitor, risedronate.

The finding in cortical bone differs from that in cancellous bone. Very little cortical bone is lost during the loss (−) phase in OX rats. It fell some 8% (Figs. 6E-G and 7B). During the restore (+) phase, new cortical bone in OX rats increased 22% (Figs. 6H and 7B). However, when PGE_2 treatment was stopped and nothing was given during the maintenance (±) phase, all but the PGE_2-induced subperiosteal bone disappeared (Figs. 6I and 7B). In contrast, when PGE_2 treatment was stopped and 1 μg risedronate per kg twice a week for 60 days was administered during the maintenance (±) phase, the PGE_2-induced subperiosteal bone and some of the subendocortical and marrow trabeculae persisted (Figs. 6J and 7B). When 5 μg risedronate per kg was given twice a week, all the PGE_2-induced bone persisted (Figs. 6K and 7B).

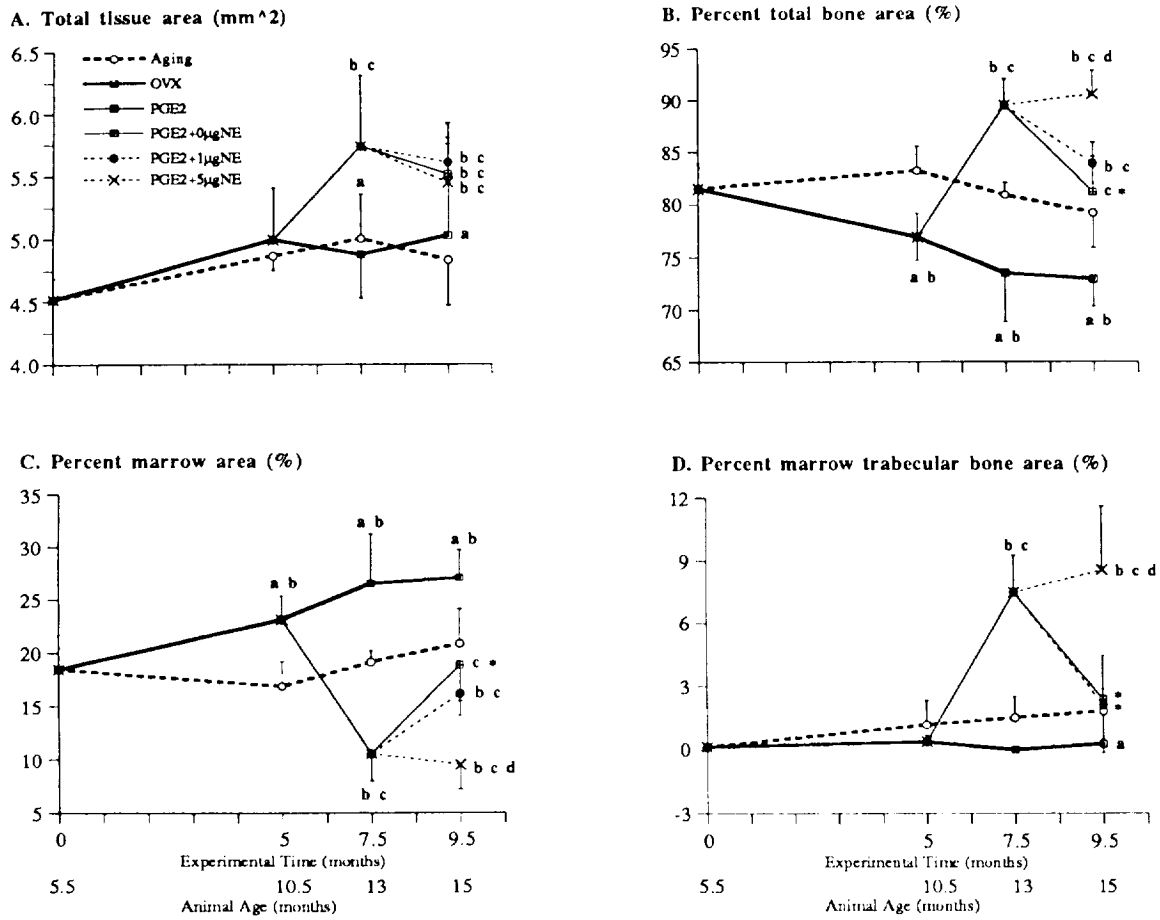

FIG. 7. Tibial diaphysis changes in aging and OX rats treated with and without PGE_2 and risedronate. Experimental time and animal age are the same as in Fig. 1. $P < 0.05$ compared to basal (a), aging (b), OX (c), PGE_2 + 0 μg NE (d), and PGE_2-treated group (*).

The study shows that new cancellous and cortical bone induced by PGE_2 can be maintained for at least 60 days after discontinuing PGE_2 by treatment with enough of the resorption inhibitor risedronate. The lower dose of risedronate was not adequate to save most of the PGE_2-induced endocortical bone.

One unforeseen finding was that administration of PGE_2 for 75 days did not completely restore the cancellous bone mass in the osteopenic proximal tibial metaphysis of the estrogen-depleted rats to age-related control levels (−38% of sham-OX control level). This was unexpected because we previously demonstrated that 30 days of 6 mg PGE_2 per kg/day restored completely bone mass to 4 month post-OX PTM.[7] We can only attribute the difference in response between the two studies to when the PGE_2 was given. PGE_2 was administered to 10-month-old rats 5 months post-OV in the current study versus 7-month-old rats 4 months post-OX in the previous study: a 3 month difference in age and 1 month more post-OX. Apparently, it is more difficult to restore bone in older rats that have been OX for a longer period even though the same amount of cancellous bone was present at 4 and 5 months post-OX.

The response to PGE_2 by the tibial shaft differed from that in cancellous bone and is more in line with what one would expect with a longer treatment period giving a greater positive bone response. In the current study, PGE_2 administration for 75 days increased the percentage of total cortical bone area by 22% over OX and 11% above aged control levels. This was much more than the 10% of OX and 6% increase in aged controls induced by 30 days of PGE_2 treatment in the previous study.[5] Apparently, in the tibial shaft the mechanostat plays a lesser role because the mean age of the tibial shafts of the two groups of rats had sufficient time to become well adapted to mechanical usage.

The 1 μg risedronate only partly maintained the extra cortical bone induced by PGE_2. Most of the endocortical bone and marrow trabeculae disappeared, but the same risedronate dose was successful in maintaining all the PGE_2-induced cancellous bone in the PTM. Three possible explanations of this difference are that (1) the bulk of the PGE_2-induced endocortical bone, especially the marrow trabeculae, is woven bone[5,11] and is more susceptible to resorption; (2) the woven bone is rapidly and poorly mineralized and may not avidly retain risedronate as well as

lamellar bone; and (3) the mechanical loading of the trabeculae in the tibial shaft is lower than for the trabeculae located in the PTM because the tibial shaft possesses a thick surrounding cortex that creates an underloaded environment favoring bone loss.[6,19]

In this study, we were able to improve our attempts to characterize the microanatomic structure of trabecular bone by incorporating the computerized method of Garrahan et al.[49] for quantitative assessment of the trabecular bone pattern. Previously we limited our analysis to trabecular area, thickness, separation, and number, after Parfitt et al.[47] The added approach to trabecular bone structure analysis proved not only to be informative, but to be more sensitive. For example, in OX rats treated with PGE_2, the parameter for node density and for the density of cortical to node increased five- to sixfold, respectively, but Parfitt's parameters increased less than a factor of 2 (area 189%; thickness 43%; separation −68%; and number 100%). Above all, both methods when automated proved to be simple and rapid.

We found it was necessary to normalize the two-dimensional structural patterns of trabecular bone to tissue and bone areas because they give different results and different meanings. For example, in OX rats, tissue-based data show decreases in node (interconnection) parameters (node density and densities of node to node, node to free end, cortical to node) but bone-based data were limited to decreases only in node density and density node to node and an increase in free and free end structures.

One of the weaknesses of the current study is in the use of a bone-seeking antiresorptive agent that is permanently retained by bone. The buildup of the bisphosphonate on bone surface, with its capacity to inhibit bone turnover, will increase bone age and could impair fatigue microdamage repair. Further, a permanent inhibition of bone turnover may hinder the further effects of an anabolic agent to increase bone mass. Possibly the use of an antiresorptive agent like calcitonin or estrogen that does not deposit in bone and possesses no long-term antiresorptive effect when discontinued may be used as a substitute for risedronate. A much safer approach would be to maintain the bone by increasing mechanical usage.[55-62]

Another weakness is that groups given risedronate but not given prior PGE_2 should have been included in the study. This would have provided invaluable information and improved our interpretation on how risedronate maintained the bone. Also, treating ovariectomized and osteopenic rats with risedronate has never been reported, and it would be interesting to determine whether the results in rats are similar to those after cyclic etidronate treatment of postmenopausal patients.[63-65]

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